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Research report

Differential modulation of lateral septal vasopressin receptor blockade in spatial learning, social recognition, and anxiety-related behaviors in rats

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Abstract

The role of lateral septal vasopressin (VP) in the modulation of spatial memory, social memory, and anxiety-related behavior was studied in adult, male Wistar rats. Animals were equipped with osmotic minipumps delivering the VP-antagonist *d*(CH₂)₅-D-Tyr(Et)VAVP (1 ng/0.5 μ l per h) bilaterally into the lateral septum (LS). Subsequently, all rats were subjected to four behavioral tests. First, animals were tested in a spatial learning paradigm (Morris water maze; 12 trials), followed by the social recognition test. A possible role for VP in anxiety-related behavior was then studied in the shock-probe burying test and the elevated plus-maze, respectively. The results showed that VP receptor antagonism impaired social recognition and reduced open-arm activity in the plus-maze, while it had no effect on spatial learning (Morris maze) and shock-probe burying behavior. The results indicate a strong task-dependent specificity of lateral septal VP functioning. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Vasopressin; Lateral septum; Social recognition; Morris water maze; Plus-maze; Shock-probe burying; Anxiety; Memory

1. Introduction

The lateral septal area is considered to be an interface between telencephalic regions such as the hippocampus and the amygdala complex on the one hand, and hypothalamic and brainstem regions on the other hand [21]. Lesion studies have demonstrated the lateral septum (LS) to be involved in the expression of fear and anxiety in two related tests, the elevated plus-maze and the shock-probe burying test [29,42]. In the elevated plus-maze, rats will normally avoid the two open arms of the maze and restrict most of their activity to both enclosed arms [33]. In the shock-probe

burying test animals will cover (i.e. bury) a stationary, electrified probe with bedding material after they experienced a mild shock from this probe [43]. Lesioning of the LS (and medial septum; MS) had an anxiolytic effect on the behavior of rats. They increased their open-arm exploration in the plus-maze and decreased their amount of burying behavior. Lateral septal lesioning has further been shown to impair spatial learning as tested in the radial maze [30]. The rats subjected to this maze showed impaired acquisition in a version of the task without intra-maze cues. In addition, working memory impairment was present throughout testing on both the cue and the place version of the task.

Neuroanatomically, the LS is known to receive an abundance of fibers containing the neuropeptide vasopressin (VP). This VP originates from neurons in the

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medial amygdala and the bed nucleus of the stria terminalis (BNST) [5,9,10,47]. The VP-containing fibers have synaptic endings in the LS, where VP exerts an excitatory action [36]. This action is presumably mediated through the postsynaptically located V_{1a} receptor subtype [32,35,39,40,44], although an additional involvement of the V_2 receptor subtype cannot be excluded [13,26,37]. The presence of this VP network suggests a modulatory role of VP in lateral septal functioning. Indeed, earlier research has implicated lateral septal VP in a diversity of behavioral and physiological functions. Since long it has been shown that locally applied VP plays a role in learning and memory, in particular in avoidance behavior [4,13,49], but also in antipyresis [6], hibernation [19], flank marking [16], and paternal behavior [50,51]. One of the best studied roles of lateral septal VP is its mediation of social memory [7,14,18,34,48]. Increasing the availability of VP in the LS improved the consolidation of conspecific recognition, whereas application of VP receptor antagonists blocked the ability of rats to recognize previously encountered conspecifics.

As mentioned before, lesion studies demonstrated the LS to be involved in anxiety-related behavior and spatial learning. Several more recent studies suggest a modulatory role for VP in these behaviors as well. Both septal V_{1a} receptor antisense and antagonist treatment were reported to have an anxiolytic effect on plus-maze behavior [25,27]. Animals treated with these substances made more entries into, and spent more time on the open arms compared with controls. Surprisingly, application of synthetic VP failed to alter plus-maze behavior [27]. To the best of our knowledge, there has been no study reporting effects of lateral septal VP manipulations on shock-probe burying behavior. Suggestions that VP might mediate spatial learning as well originate from studies on long-term potentiation (LTP [17,46]), an experimental model thought to underlie the cellular processes of memory formation. It was shown that VP maintained LTP and facilitated excitatory transmission in septal brain slices, whereas this maintenance was prevented by an antagonist of the V_{1a} receptor [22,45,46]. However, *in vivo* experiments using microdialysis have shown that VP administration into the mediolateral septum impaired spatial learning, while VP antagonism left acquisition behavior undisturbed [11].

In summary, the scarce and sometimes contradictory literature suggests a modulatory role of VP in anxiety and spatial learning. Therefore, the aim of the present experiment was to further explore the modulatory role of VP in lateral septal functioning in animals subjected to a number of tasks. Adult male rats were equipped with osmotic minipumps delivering a VP antagonist locally into the LS and they were subjected to four behavioral tests. The V_2/V_1 antagonist $d(\text{CH}_2)5\text{-D-}$

Tyr(Et)VAVP was used to block all VP receptors (presumably) present in this area. First, the animals were tested in a spatial learning paradigm (the Morris water maze [31], followed by the social recognition test, because VP's role in it is one of the best documented. Finally, to confirm and expand the knowledge on VP in anxiety-related behavior, the shock-probe burying test and the plus-maze test were included. The results of this array of behavioral tests performed with the same animals may also allow us to give an answer to the question whether lateral septal VP acts task specific or that it exerts its action through a common mechanism, independent of the task.

2. Materials and methods

2.1. Animals

The subjects were male Wistar rats (± 350 g) obtained from our own breeding facilities. Initially, animals were housed in Macrolon type I cages in groups of five to eight animals. After surgery they were housed individually in standard cages ($20 \times 30 \times 15$ cm) in a sound attenuated room. They had free access to water and lab chow on a 12:12 h light–dark cycle (lights off at 08:30 h) at a temperature of 19–21°C. Always present was a small 15-W bulb providing some very dim light.

2.2. Surgery

A total of 24 animals received bilateral brain cannulas placed in the lateral septum for infusing the VP antagonist using osmotic minipumps. This method reduced stress of handling to a minimum during behavioral testing. Briefly, rats were halothane-anesthetized and placed in a stereotaxic device. They received two 25-gauge stainless steel guide cannulas (implantation coordinates: 0.2 mm posterior to Bregma, each 1.0 mm media-lateral, depth of 2.2 mm from dura, extending 2.8 mm above dura). The guide cannulas were secured to the skull with three stainless steel screws and dental cement. A short wire (diameter 0.12 mm) closed the guides and bone wax was applied to cover the cannulas. The incision was closed and rats were allowed to recover for at least 6 days. At 1 day before implantation, the osmotic minipumps (Alzet, model 2002, 0.5 $\mu\text{l/h}$) were filled with either saline or the VP antagonist. The V_2/V_1 antagonist $d(\text{CH}_2)5\text{-D-Tyr(Et)VAVP}$ (Sigma, V4253 [28,38]) has been shown to be equally potent as the most commonly used V_1 antagonist $d(\text{CH}_2)5\text{-Tyr(Me)AVP}$ [13]. Based on studies of the group of Landgraf [11,13,27], the antagonist was administered bilaterally in a concentration of 2 ng/ μl , enough to ensure a total receptor blockade. A 10-cm length of

polyethylene tubing was filled with the corresponding fluid and attached to the minipump. An air bubble was created at the free end of the tubing. The pumps were placed in small jars filled with saline and with tubings extending above the fluid level, at 37°C overnight. In this way the pumps could reach a stable pumping rate and the air bubble allowed a check of the proper functioning of the pumps. The rats were halothane-anesthetized and received two minipumps placed subcutaneously. The tubings were length-adjusted and attached to the injector cannulas (0.15 mm inner diameter), which were placed into the guide cannulas after removal of the bone wax and steel wire. This procedure ensured bilateral placement of the cannula tips into the lateral septum, 4.6 mm below the dura mater. The assembly was secured to the skull with dental cement and the incisions were closed again. A total of 13 animals were infused with the antagonist *d*(CH2)5-D-Tyr(Et)VAVP, 11 with saline. All animals were allowed 2 days of recovery before behavioral testing was started.

2.3. Morris maze

Behavioral testing commenced with Morris maze swimming performed in a polyester circular pool (diameter: 140 cm, height: 35 cm) with a featureless black inner surface. It was located in a large observation room, illuminated by three red light tubes in order to maintain a reversed light–dark cycle. Positioned near the experimenter and the computer system was a 15-W bulb providing some dim light. Swimming behaviour was registrated by a computerized video imaging analysis system (EthoVision, Noldus Information Technology, Wageningen, Netherlands) with a camera hanging over the pool. Computer screen and tv-monitor were light attenuated and computer beeps were omitted from the program during the testing period. The pool was filled to a height of 25 cm with water of 27°C ($\pm 1^\circ\text{C}$). The pool was divided into four (imaginary) quadrants named A–D. The hidden escape platform (diameter: 9 cm) was submerged 2 cm below the water surface in quadrant A, invisible at water level. Several external, constant cues surrounded the pool.

Animals were brought to the observation room 1 h prior to testing with cages covered light-tightly to reduce disturbances of the light–dark cycle. They received three trials each day for 4 days, reaching a total of 12 trials. The intertrial time was set to 1 h. During initial acquisition, the escape platform, in quadrant A, was placed 12 cm from the rim of the pool. From trial 10 on (day 4) the platform was placed in the opposite quadrant (C), 24 cm from the rim. Rats were gently placed in the water facing the centre of the pool, while the starting position was varied pseudo-randomly over the trials. The rats were allowed 2 min to find the

escape platform; if it was not found within this time, the animals were placed on the platform for 30 s. After the last trial they were returned to their housing room.

2.4. Social recognition

Social recognition was performed in the housing room under red and dim light conditions. Testing took place two days after the last Morris maze trials and between 13:00 and 17:00 h. Male juveniles, 25–30 days old and housed in groups of six, were used as social stimuli. The juveniles were placed individually 2 h prior to testing. All adult rats ($n = 24$) were tested in their home cage. An initial 5-min exposure to a juvenile was followed by a second exposure to the same juvenile after 30 min. Juveniles were kept individually between both exposures. All social encounters were video-taped with no experimenter present in the room during the test. Social investigatory behavior was scored by a trained observer, unaware of the test settings. Behavior directed towards the juvenile mostly consisted of anogenital sniffing, close following, and pawing. The amount of time animals spent on these behaviors (investigation time) gives a measure of social recognition.

2.5. Shock-probe bury

Immediately after the social recognition test animals were placed in perspex cages (25 \times 25 \times 30 cm, 3 cm of bedding material) under conditions as mentioned before. On one wall, 2 cm above the bedding, was a small hole through which the shock-probe could be inserted during testing. The Teflon shock-probe (6.5 cm long, 1 cm in diameter) was wrapped with two wires through which an electric current of 1.5 mA could be administered. The rats were left undisturbed for three nights (no probe present) before testing started. The behavioral testing was performed between 09:00 and 13:00 h. The continuously electrified probe was inserted into the cage. Upon touching the probe the animal received a brief electric shock. Following the first shock, the duration of time each rat spent pushing bedding material towards and on top of the probe (burying) was measured for 10 min. In addition, the number of shocks the rat received and behavioral components as immobility, rearing, exploring and grooming were also monitored during a session. All rats and cages were coded so the observer was unaware (both in shock-probe and plus-maze testing) of the treatment each animal received.

2.6. Plus-maze

Following the shock-probe bury test, but with at least 3 h in between, the rats were tested for their level of anxiety in the plus-maze (between 14:00 and 17:00 h). The apparatus was a black, wooden, plus-shaped

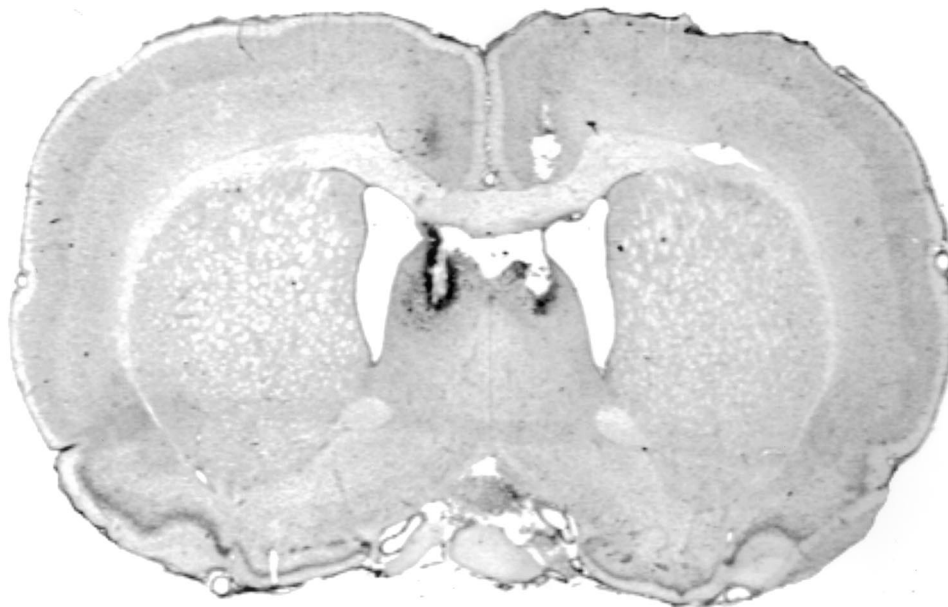


Fig. 1. Digital photomicrograph of a coronal section displaying the localization of the tips of the minipump-connected brain cannulas (bilateral) in the lateral septal area.

maze which was placed in a separate, dimly lit room. The maze was elevated to a height of 60 cm and each of the four arms measured 50×10 cm. The open arms had a rim of 1 cm and both closed arms had walls (50 cm) with an open top. The observer placed one rat in the test room (covered cage) and after 1 min the rat was placed in the center of the maze. During the 5-min test several behaviors were measured: time spent on the open arms, closed arms, and central platform; number of entries on open and closed arms; number of center-crossings; number of times animals were showing only their head. A relative measure for open-arm activity was calculated by dividing the time spent on the open arms by the total time spent on both open and closed arms ($O/O + C$). An entry was defined as all four paws being on the arm. The maze was cleaned after each animal was tested.

2.7. Cannula placement

At 2 weeks after the minipump implantation all animals were transcardially perfused with 50 ml of saline followed by 200 ml of 4% paraformaldehyde (pH 7.4, 0.01 M phosphate buffer). The cannula-assembly was carefully removed from the skull and checked for irregularities. Brains were dissected and placed in 30% sucrose overnight at room temperature. Cryostat sectioning (30 μ m) was followed by light microscopic examination of cannula placement.

2.8. Statistics

Results are expressed as means \pm S.E.M. The morris

maze results were analysed using two-way ANOVA with repeated measures (treatments \times trial, between- and within subject factors, respectively). The same procedures were used for the social recognition data (treatments \times exposures). These were followed by individual interval and treatment comparisons using post hoc paired sample *t*-tests. Results obtained during shock-probe and plus-maze testing were analysed using the Student's *t*-test.

3. Results

Histological verification of the brains of the cannulated rats revealed improper placement in three animals. Fig. 1 illustrates a representative coronal brain section showing the tracks of correctly placed cannulas in the LS. The minipump tubing had been obstructed in two animals during the experiment, leaving nine saline-treated and ten VP antagonist-treated rats to be included in Morris maze, shock-probe, and plus-maze analysis. During social recognition one rat showed severe aggression towards the juvenile and was discarded from statistical analysis. Incomplete video registration during recognition testing further reduced the number of saline- and VP antagonist-treated animals to eight and nine, respectively.

3.1. Morris maze

All animals received 12 trials in the water maze, nine trials to acquire the test followed by three additional trials to study the effect of replacing the fixed platform.

The results are illustrated in Fig. 2. On the first trial all rats swam approximately 15 m within or until 2 min had passed. On trial 4 (first trial on 2nd day) saline-treated animals again needed about 15 m to reach the escape platform, but they rapidly adjusted in the following trials. All animals reached asymptotic performance levels within nine trials. Replacing the platform on trial 10 caused an increase in travelled distance in both treatment groups. In the following trials they were able to quickly adjust to the new situation. Statistic analysis revealed no significant differences in travelled distance between both treatments at any stage during the acquisition or after replacing the platform. ANOVA-testing showed that trial (within-subject factor) was a significant factor during the first nine trials ($F_{8,144} = 12.99$, $P < 0.01$) and the last three trials ($F_{2,36} = 8.92$, $P < 0.01$). This confirms that both groups were able to learn and to adjust to the water maze task.

Shown in the upper right section of Fig. 2 are the results of the time animals spent in quadrant A (original position of the escape platform) during trial 10. No significant differences were found in this parameter. Average swimming speed (cm/s) during spatial learning in the Morris water maze is illustrated in Fig. 3. Although there is some variation in speed over the trials with an average of approximately 17 cm/s, no significant group differences could be detected. Replacing the escape platform induced a mild increase in speed, but it was adjusted to the average speed on the remaining two trials.

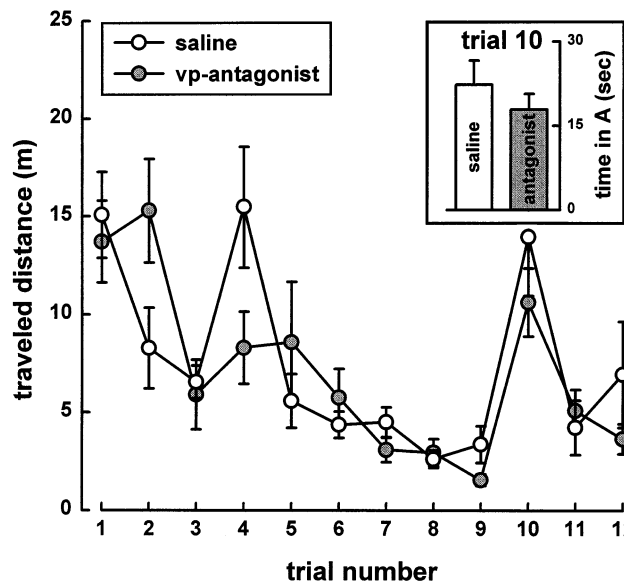


Fig. 2. Results of spatial learning in the Morris water maze. There are no significant differences in travelled distance between both treatments at any stage during the acquisition or after replacing the platform on trial 10. The results showed that both groups were able to learn and to adjust to a new situation. Shown in the upper right corner are the results of the time animals spent in quadrant A (original position of the escape platform) during trial 10. No significant differences were found in this parameter (saline-treated group: $n = 9$; VP antagonist-treated group: $n = 10$).

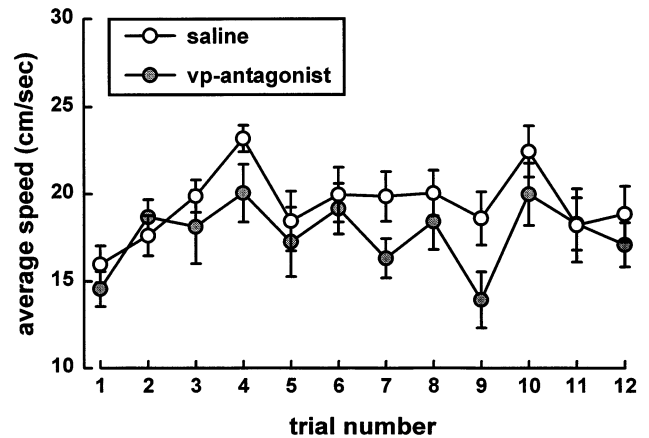


Fig. 3. Average swimming speed (cm/s) during spatial learning in the Morris water maze. Although there is some variation in speed over the trials with an average of approximately 17 cm/s, no significant group differences could be detected. Replacing the escape platform induced a mild increase in speed, but it was adjusted to the average speed on the remaining two trials.

cm/s, no significant group differences could be detected. Replacing the escape platform induced a mild increase in speed, but it was adjusted to the average speed on the remaining two trials. Other measures included in the analysis of swimming behavior were escape latency to the platform and time spent in either of the four quadrants (both absolute and in percentage of the total amount of time). None of these measures revealed a significant difference in performance between the treatment groups.

3.2. Social recognition

Animals were tested for social recognition using two successive exposures to the same juvenile with a 30-min interval. The results for this behavioral paradigm are illustrated in Fig. 4. During the first exposure to a juvenile both saline- and antagonist-treated groups showed comparable levels (± 180 s) of investigation. During the second exposure the saline-treated group decreased their amount of time studying the juvenile to approximately 140 s. Instead, the group treated with VP antagonist increased its investigatory behavior compared with the first exposure to just above 200 s. Analysis of variance showed a significant effect of treatment, $F_{1,15} = 8.92$, $P < 0.01$, but no effect of exposure due to the diverging reactions of both treatment groups. The interaction (treatment by exposure) proved highly significant again, $F_{1,15} = 14.99$, $P < 0.01$, emphasizing the different reactions during the second exposure. Post hoc *t*-testing confirmed the decrease and increase in investigation observed in the saline- and antagonist-treated group, respectively ($P < 0.05$, two-

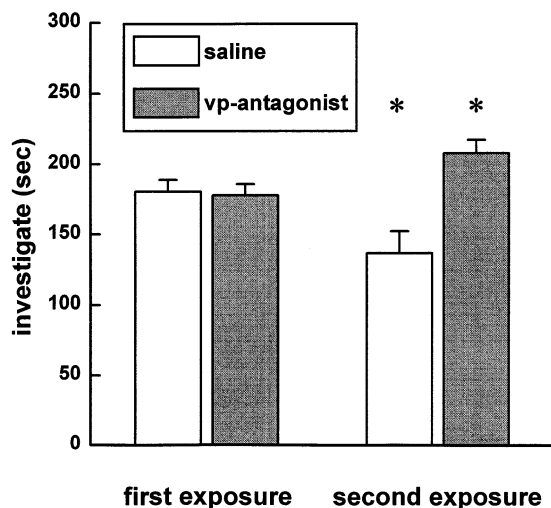


Fig. 4. Animals were tested for social recognition in two successive exposures to the same juvenile with a 30-min interval. During the first exposure both the saline- and antagonist-treated group spent equal amounts of time investigating the juvenile. During the second exposure the saline-treated group showed a reduction in investigation time. Instead, the group of animals treated with VP antagonist increased investigatory behavior when compared with the results of the first exposure (* $P < 0.05$, two-tailed; saline-treated group: $n = 8$; VP antagonist-treated group: $n = 9$).

tailed). None of the other behaviors observed during the paradigm (e.g. grooming, rearing, immobility) were significantly different over all test settings.

3.3. Shock-probe bury

Fig. 5 indicates that both treatment groups spent equal amounts of time burying, exploring, and rearing, irrespective of the treatment. Moreover, in no other measure, including the number of probe contacts and

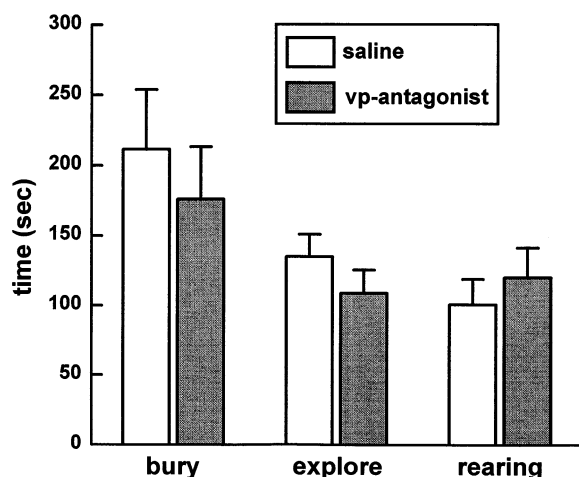


Fig. 5. Illustrated are results from three major behavioral components observed during the 10-min shock-probe burying test. None of these measures showed significant differences between the saline ($n = 9$) and antagonist-treated group ($n = 10$).

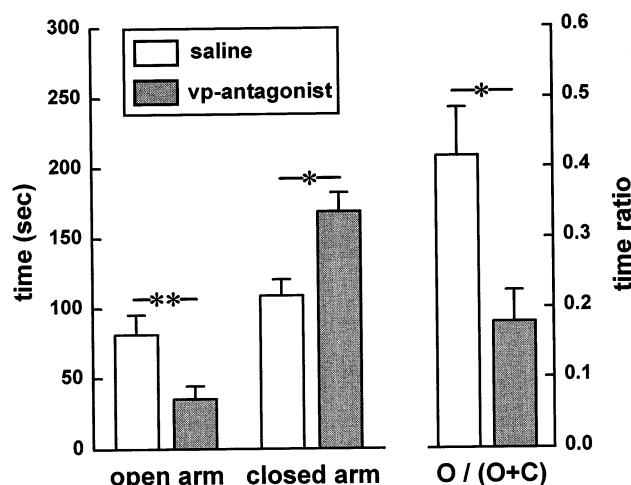


Fig. 6. Anxiety measurement using the elevated plus-maze. The VP antagonist-treated group exhibited lower levels of open-arm activity and increased its time spent on the closed arms (left panel). This resulted in a significantly lower time ratio (right panel) compared with the saline-treated group (* $P < 0.01$, two-tailed; saline-treated group: $n = 9$; VP antagonist-treated group: $n = 10$).

immobility scores, we were able to detect any significant effect of VP antagonist treatment.

3.4. Plus-maze

Fig. 6 illustrates the time-related results of the plus-maze experiment. The group of VP antagonist-treated animals exhibited lower levels of open-arm activity ($P < 0.05$) and increased its time spent on the closed arms ($P < 0.01$), resulting in a significantly lower time ratio (Fig. 6, right panel, $P < 0.01$) compared with the saline-treated group. In addition, the infusion of the antagonist significantly reduced the number of entries on the open arm as opposed to the controls ($P < 0.01$, see Fig. 7). However, there were no group differences in the number of closed arm entries. The right panel of Fig. 7 shows that the saline-infused group made significantly more central-platform crossings ($P < 0.05$) than the antagonist-treated group.

4. Discussion

We investigated the effect of a locally applied V_2/V_1 receptor antagonist ($d(CH_2)5$ -D-Tyr(Et)VAVP) on the behavior of rats, all subjected to the same behavioral paradigms. This treatment impaired social recognition and reduced open-arm activity in the plus-maze, while it had no effect on spatial learning (Morris water maze) and on shock-probe burying behavior.

The use of subtype-selective VP radioligands suggests that the majority of VP binding in the brain is to the V_{1a} receptor. The presence of this receptor subtype in

the septum is well established and demonstrated by a wide variety of techniques [32,35,39,40,44]. The V_2 receptor has only recently been demonstrated in the brain [20,23]. Although a functional V_2 -type receptor has been suggested to exist in the septum as well [13,26,37], its presence there seems too low to be detected. The use of a combined V_2/V_1 antagonist in the present experiment ensured blockade of both types of VP receptors in the LS. The dosage of administration was adapted from recent experiments studying VP functioning in the LS and it is assumed that this dosage results in a total blockade of both receptor types [11,13,26,27].

Studies on the specific role of the neuropeptide in spatial memory are sparse, although VP has frequently been implicated in learning and memory (for reviews, see [15,49]). Lesion studies have indicated a function for the LS in spatial learning, since disruption of this area impaired the acquisition of both place and cue versions of the radial maze task [30]. In the present experiment we found no effect of VP receptor blockade on either the acquisition of the test or on recalling the platform position after replacement (see Fig. 2, trial 10). These data are in accordance with the results of Engelmann et al. [11], who have used microdialysis techniques to infuse VP or a selective V_1 receptor antagonist ($d(\text{CH}_2)_5\text{-Tyr(Me)AVP}$) into the mediolateral septum during Morris maze spatial learning. They reported that place learning was impaired by administration of exogenous VP, whereas treatment with the antagonist had no effect on acquisition of the test when compared

with control animals. Although it does not exclude a role for VP, the present study confirms that endogenous VP in the LS is not causally involved in spatial learning, at least not via the V_1 and V_2 receptor subtypes [11,15].

The present results seem to be consistent with the literature on the social recognition paradigm [7,14,18,25,34,48]. Normally, there is a reduction in investigation time between two successive exposures to the same juvenile. This reduction is regarded as being the result of (social) recognition and it is considered a form of short-term memory. However, when the inter-exposure time exceeds approximately 60 min, investigation time of the second exposure equals that of the first one and there is no recognition. Application of exogenous VP facilitates social recognition: the inter-exposure time can be increased up to 120 min without affecting recognition. Administration of VP antagonists into the septum blocks this form of social memory. In the present experiment, saline-treated rats showed the anticipated decrease in investigatory behavior when they were confronted with a familiar juvenile (Fig. 4). Infusion of the VP antagonist induced the adult animal to spend even more time investigating the juvenile on a second exposure, indicating a failure to recognize it. This increase has been observed by other investigators as well, using acute injections to apply the substances, but has never been discussed as such [3,8,12,34]. Further studies are necessary to investigate whether the additional blockade of the (putative) lateral septal V_2 receptor is responsible for the increased investigation time during the second exposure as observed in the present experiment.

There is a discrepancy between the present results of social recognition and a study by Bluthé and Dantzer [2], who used Accurel collodion mini-devices to chronically release the VP antagonist $d\text{PTyr(Me)AVP}$ into the cerebro-spinal fluid. They showed that intact male rats, treated with the antagonist for at least three weeks, did recognize juveniles exposed to them for the second time at 30 min (and even at 120 min) after the first exposure, a result opposite to that found in the present experiment and in studies which use acute injections. Bluthé and Dantzer [2] argued that the prolonged antagonist treatment induced a transition from a (lateral septal) VP-dependent form to a VP-independent form of social memory. This transition can also be observed when the performance of intact males is compared with their performance after they are gonadectomized. A number of studies (for review, see [18]) have shown that gonadectomized rats (and females) are able to recognize juveniles for a longer interval, about 120–180 min, and do so without lateral septal VP participating in it. The VP network of the LS is known to be and androgen-dependent [9,10] and castration of males dramatically decreases the amount of VP in this area over a period

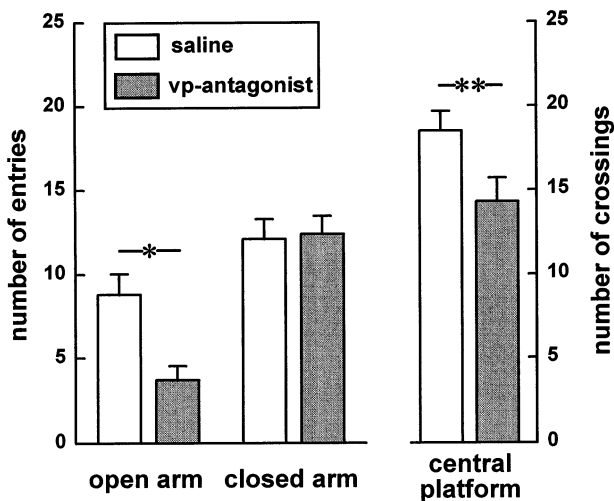


Fig. 7. Number of entries on both types of arms and the number of central platform crossings on the elevated plus-maze. The saline-treated group made significantly more entries onto the open arm compared with the antagonist-treated group (* $P < 0.01$, two-tailed). Both groups made an equal number of entries into the closed arms of the maze. This resulted in a concomitant difference in the central platform crossings: the saline-treated group showed a higher number of crossings when compared with their antagonist-treated counterpart (** $P < 0.05$, two-tailed).

of at least 10 weeks. For social recognition, these animals then 'switch' to another neural system for proper performance. Chronic blockade of lateral septal VP functioning seems to induce this transition as well. In the present experiment however, the data are in accordance with results from acute injections. We suggest that the period of antagonist treatment (8 days as opposed to at least 21 days) has been too short to induce the transition from a VP-dependent to a VP-independent form of social recognition. More studies are necessary to confirm this suggestion.

Lesioning of the lateral septum reduces the amount of burying in the shock-probe test [29,42], suggesting a decreased anxiety level. To the best of our knowledge, there are no studies which have investigated the role of lateral septal VP on the behavior of rats in this test. We anticipated an anxiolytic effect of VP receptor blockade based on results obtained from elevated plus-maze experiments [25,27]. The present results however, suggest that endogenous VP is not involved in the initial response to a mild shock, since application of the combined V_2/V_1 receptor antagonist did not influence burying behavior in any way (see Fig. 5). Engelmann et al. [13] have shown that septal administration of a V_1 or a V_2/V_1 antagonist impaired performance in a conditioned active avoidance test (pole-jumping). This indicates that endogenous VP may be involved in the acquisition and storage of information in situations of fear and anxiety. Given this, it may be tempting to study the effect of the peptide in more detail in the conditioned paradigm of the shock-probe test. This version of the test, in which the animal is re-exposed to the (un electrified) probe at a later time, i.e. 24 h later, might prove to be under vasopressinergic control.

In the elevated plus-maze, no significant differences were found in the number of entries into closed arms, a typical indicator of general locomotor activity (Fig. 7). This means that differences found in time spent on open and closed arms is not caused by a difference in overall activity. Open-arm activity in the plus-maze was reduced in rats treated with the VP antagonist. This latter observation is in contrast with recent studies reporting anxiolytic effects of V_1 receptor manipulation. Antisense treatment aimed at this VP receptor reduced anxiety, suggested to be caused by the reduction in the number of available ligand-receptor sites [25]. More strikingly, Liebsch et al. [27] reported that animals treated with V_1 receptor antagonist ($d(\text{CH}_2)_5\text{-Tyr(Me)AVP}$) spent more time on and made more entries into the open arms of the plus-maze, indicating reduced anxiety. Although their microdialysis technique and the present infusion method both were aimed at infusing a VP receptor antagonist into the septum, the behavioral effects are completely opposite. It is hard to discern what factor may underlie this difference. The additional blockade of V_2 subtype receptors might have

contributed to this difference, but this is unlikely. Both the V_2/V_1 antagonist $d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$ and the selective V_1 antagonist $d(\text{CH}_2)_5\text{-Tyr(Me)AVP}$ have been shown to be equally potent and to influence pole-jumping behavior in the same manner [13]. Furthermore, our results in the social recognition test and the Morris maze are completely comparable to studies utilizing only the latter antagonist, which suggests identical action of both substances [11,25,34].

The chronic treatment may have been a factor influencing the outcome of the test. Liebsch et al. [27] administered the antagonist by means of microdialysis, 30 min prior to testing. Four days of chronic antisense treatment, resulting in a reduced binding of VP to the V_1 receptor in the septum, showed to have an anxiolytic effect on the rat's behavior in the plus-maze as well [25]. In the present experiment, animals were tested on the 11th day after start of the infusion. However, at this moment we have no firm indications that the chronicity of the treatment is responsible for the anxiogenic effect as opposed to the earlier mentioned studies. Additional experiments are necessary to study this aspect in more detail.

The opposing effects were not caused by the fact that the shock-probe test preceded the plus-maze paradigm with only a few hours in between. It is feasible to suggest that receiving shocks might raise the anxiety level of the rats, which would effect open-arm activity. Therefore, in a follow-up study, we operated and equipped an additional 20 animals with osmotic minipumps in an identical way and time schedule as described here. They were tested only in the plus-maze without any behavioral testing preceding it. The results from this additional, independent plus-maze test showed the same anxiogenic effect of the VP antagonist as in the present experiment (data not shown).

Still, one may wonder why two tasks, which, at first glance seem to test the same modality of behavior (anxiety), are differentially regulated by VP. It has been suggested that both tests may differ in what is required of the animals confronted with the tests. The plus-maze is thought to test treatment effects on exploration, whereas the shock-probe test measures effects on neophobia and aversion (here: unfamiliar probe in home cage) [1,41]. The behavior evoked by this variation in the 'character' of both tests may be differentially regulated in the LS.

Based on the results from the present and previous experiments, and if we assume that the method used here resulted in a complete VP receptor blockade, we may conclude that there is a strong specificity in the role of VP modulation depending on the task an animal is subjected to. Although this aspect of VP function has been mentioned earlier [15,24], we feel that the surplus value of this experiment is that the effect of one treatment in the same animal was tested in several

paradigms, ruling out treatment differences between successive tests. As expected, social memory was impaired by VP antagonism. The same treatment reduced open-arm activity in the plus-maze, but it had no effect on spatial memory and shock-probe burying.

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